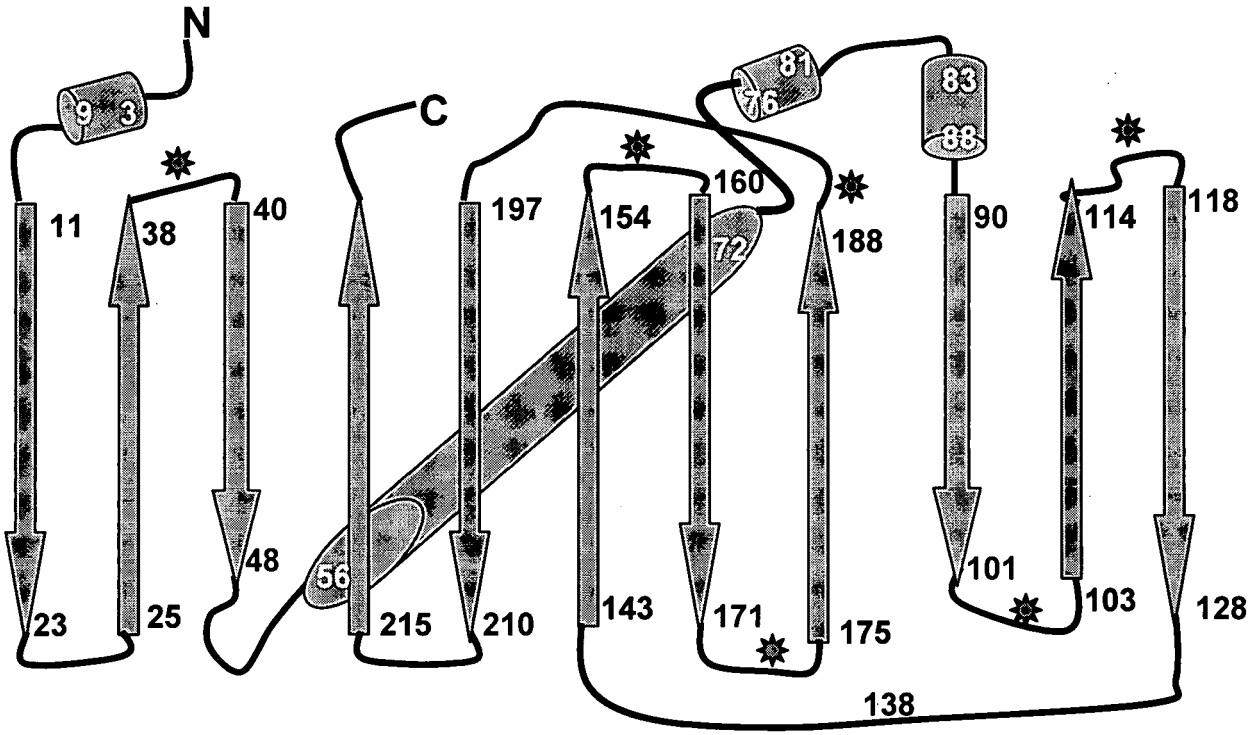
Fig. 1a



Aequorea Victoria Green Fluorescent Protein (GFP)

✸ Loops highlighted by asterisks designate alternative fragmentation sites that are the subject of the invention, comprising amino acid residues 38-40 (region 1); residues 101-103 (region 2); residues 114-118 (region 3); residues 154-160 (region 4); residues 171-175 (region 5); and residues 188-190 (region 6).

Fig. 1b

3-dimensional structure of A. victoria GFP showing the positions of specific amino acids at 6 alternative fragmentation sites

1. Select a fluorescent protein and its corresponding DNA sequence
2. Create fragments and mutant fragments by:

   A. Rational design, followed by genetic engineering or oligo-nucleotide synthesis; and/or

   B. Random or directed fragmentation of full-length DNA molecule
3. Create 5' or 3' fusions of fragments (F1,F2) with genes encoding other proteins (A,B) in expression vectors
4. Co-transfect fragment pair fusions into cells; allow expression of fusion proteins A,B
5. Measure fluorescent signal, e.g. by fluorometry, FACS, or microscopy

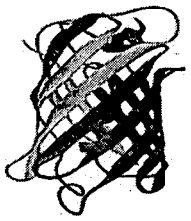


Fig. 2

Fig. 3

24 Hour Expression in HEK293E Cells (1.2 sec exposure)

Fig. 4

Examples of PCAs based on alternative fragmentation sites of YFP

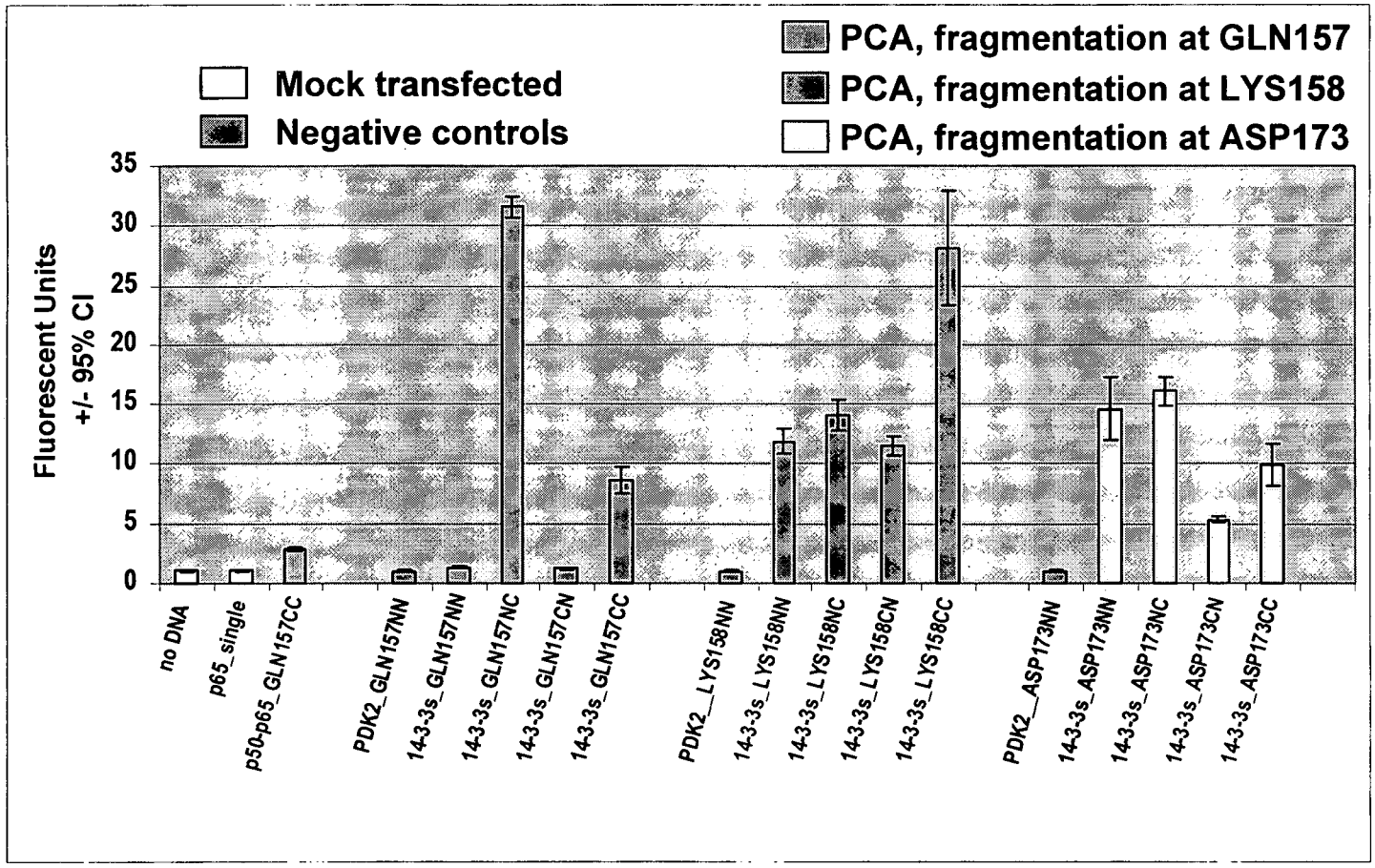
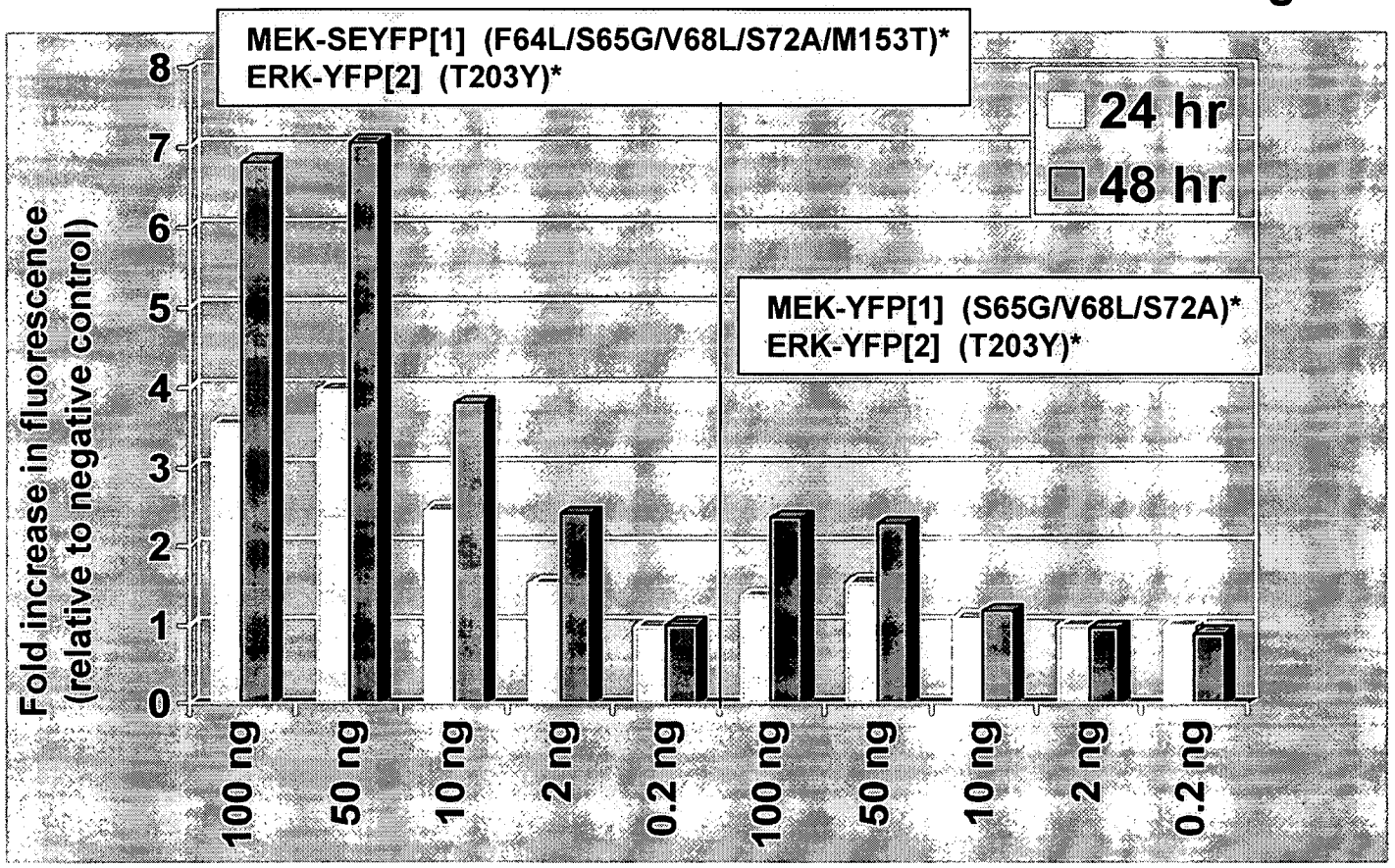


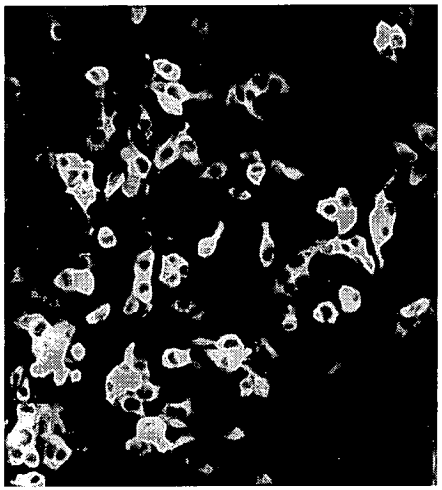
Fig. 5



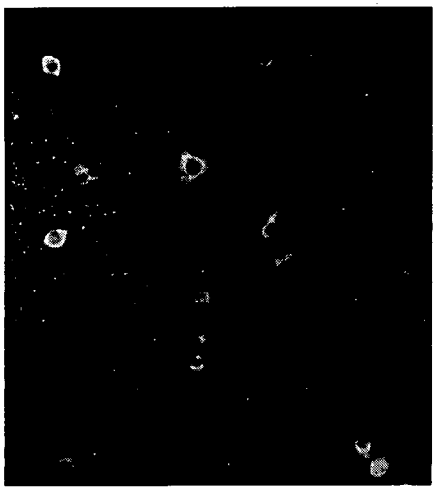
* Note: amino acid changes are designated relative to wild-type GFP

Fig. 6

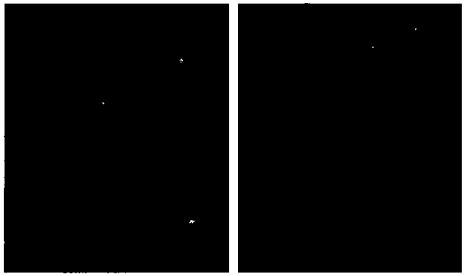
a. MEK-SEYFP[1] (F64L/S65G/V68L/S72A/M153T)*
ERK-YFP[2]-T203Y*



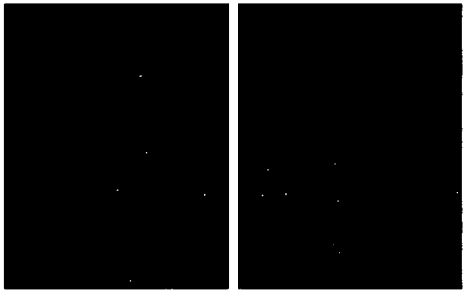
b. MEK-YFP[1] (S65G/V68L/S72A*)
ERK-YFP[2] (T203Y*)



c. Single fragment control:
Pdk2-YFP[1] (F64L/S65G/V68L/S72A)



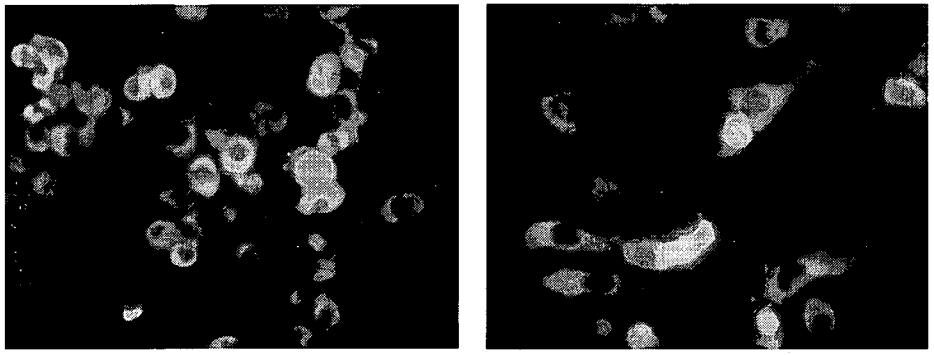
d. Single fragment control:
Pdk2-YFP[2] (T203Y)



** Note: amino acid changes are designated relative to wild-type GFP*

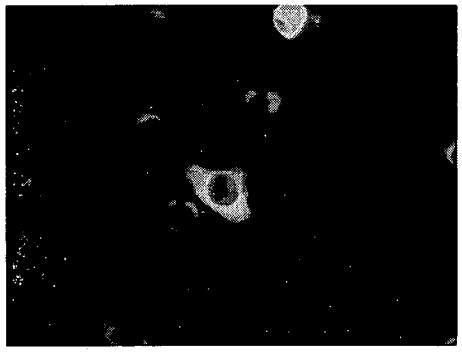
Fig. 7a

An intense fluorescent PCA (IFP PCA) based on mutant fragments of YFP



100 ng DNA (19 ms)

10 ng DNA (19 ms)



1 ng DNA (95 ms)

0.1 ng DNA (950 ms)

Results shown are for MEK-IFP[1] + IFP[2]-ERK

IFP[1]: F46L/F64L/S65G/V68L/S72A/M153T
IFP[2]: V163A/S175G/T203Y
Note: amino acid changes are designated relative to wild-type GFP

Fig. 7b.

Enhanced PCA signals based on mutant fragments:
IFP (SEYFP-F46L) versus EYFP

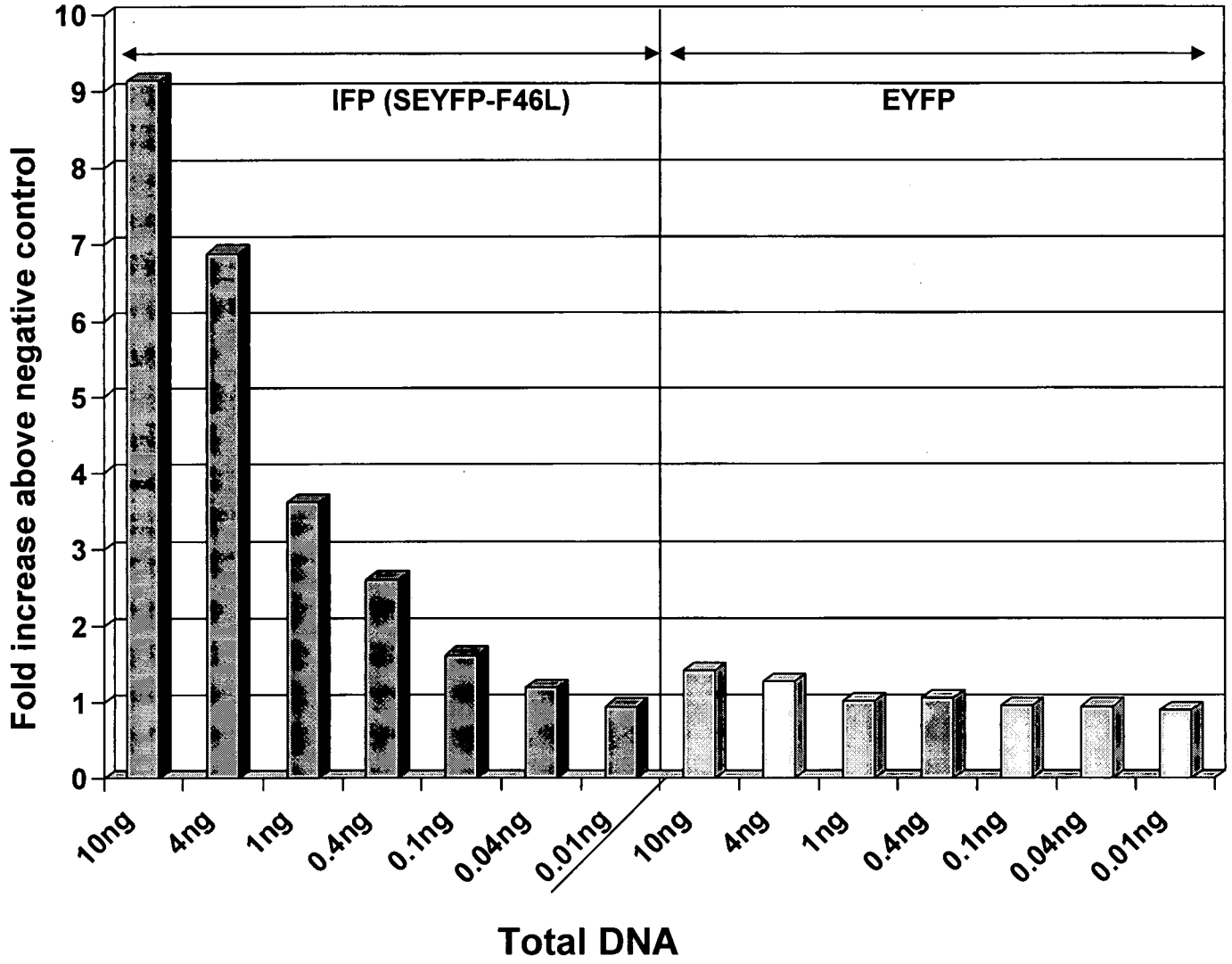


Fig. 8
High-content assays based on IFP PCA:
cytokine-dependent translocation of p65/p50

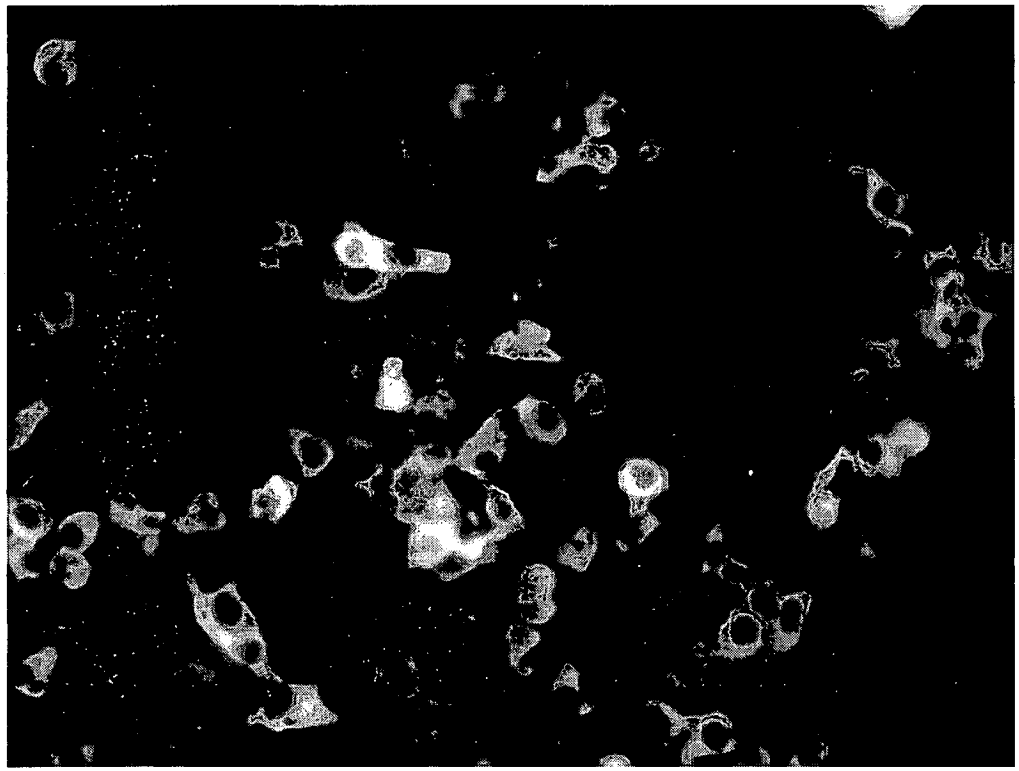
Fig. 9 .

Spectrally shifted (blue) PCAs based on mutant fluorescent protein fragments (excitation= 436 nm, emission= 480 nm)

14-3-3σ/14-3-3σ | p65/p50 | Negative Control

Fig. 10.
Multi-color protein-fragment complementation assays (PCA)

P50-CFP[1] +
P65-CFP[2] +
IκBα-YFP[1]



CFP channel
Excitation= 426-446 nm
Emission= 460-500 nm

FITC channel
Excitation=460-500 nm
Emission= 505-560 nm